IT IS CLAIMED:

1. A β-secretase enzyme protein purified to apparent homogeneity.

2. The purified ß-secretase enzyme protein of claim 1, wherein the enzyme has been purified sufficiently so that its activity in cleaving the 695-amino acid isotype of ß-amyloid precursor protein (ß-APP) between amino acids 596 and 597 thereof is at least 10,000-fold greater than an activity exhibited by a solubilized but unenriched membrane fraction from human 293 cells.

3. The purified ß-secretase enzyme protein of claim 1, characterized by a specific activity of at least about 0.2 x 10^5 nM/h/μg protein in an MBP-C125sw substrate assay.

4. The purified β-secretase enzyme protein of claim 3, wherein said specific activity is at least 1.0 x 10^5 nM/h/μg protein.

5. The purified ß-secretase enzyme protein of claim 1, wherein said protein is fewer than 450 amino acids in length, comprising a polypeptide having the amino acid sequence SEQ ID NO: 70 [63-452].

6. The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 70 [63-452].

7. The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 69 [63-501].

8. The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 67 [58-501].

9. The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 68 [58-452].

10. The purified protein of claim 5, wherein said protein comprises a polypeptide having the amino acid sequence SEQ ID NO: 58 [46-452].

11. The purified protein of claim 10, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 74 [22-452].

12. The purified protein of claim 10, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 58 [46-452].

13. The purified protein of claim 10, wherein said protein is characterized by an N-terminus at position 46 with respect to SEQ ID NO: 2 and a C-terminus between positions 452 and 470 with respect to SEQ ID NO: 2.

14. The purified protein of claim 10, wherein said protein is characterized by an N-terminus at position 22 with respect to SEQ ID NO: 2 and a C-terminus between positions 452 and 470 with respect to SEQ ID NO: 2.

15. The purified protein of claim 1, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 43 [46-501].

16. The purified protein of claim 1, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 66 [22-501].

17. The purified protein of claim 1, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 2 [1-501].

18. The purified protein of claim 1, wherein said protein has an N-terminal residue corresponding to a residue selected from the group consisting of residues 22, 46, 58 and 63 with respect to SEQ ID NO: 2 and a C-terminus selected from a residue between positions 452 and 501 with respect to SEQ ID NO: 2.

19. The purified protein of claim 18, wherein said C-terminus is between residue positions 452 and 470 with respect to SEQ ID NO: 2.

20. The purified protein of claim 1, wherein said protein is isolated from a mouse.

21. The protein of claim 20, wherein said polypeptide has the sequence SEQ ID NO: 65.

22. The purified protein of claim 1, wherein said protein is produced by a heterologous cell.

23. A crystalline protein composition formed from a purified β-secretase protein.

24. The crystalline protein composition of claim 23, wherein said purified protein is characterized by a binding affinity for the β-secretase inhibitor substrate P10-P4'sta D→V which is at least 1/100 of an affinity exhibited by a protein having the amino acid sequence SEQ ID NO: 43 [46-501], when said proteins are tested for binding to said substrate under the same conditions.

25. The crystalline protein composition of claim 23, wherein said composition is formed from a protein having a sequence selected from the group consisting of SEQ ID NO: 66 [22-501], SEQ ID NO: 43[46-501], SEQ ID NO: 74 [22-452], SEQ ID NO: 43 [46-452], and SEQ ID NO: 71 [46-419].

26. The crystalline protein composition of claim 23, wherein said composition is formed from a protein having a sequence selected from the group consisting of SEQ ID NO: 2 [1-501], SEQ ID NO: 59[1-452], and SEQ ID NO: 60 [1-420].

27. The crystalline protein composition of claim 23, wherein said composition is formed from a protein having an N-terminal residue corresponding to a residue selected from the group consisting of residues 22, 46, 58 and 63 with respect to SEQ ID NO: 2 and a C-terminus selected from a residue between positions 452 and 501 with respect to SEQ ID NO: 2.

28. The crystalline protein of claim 27, wherein said C-terminus is between residue positions 452 and 470 with respect to SEQ ID NO: 2.

29. The crystalline protein composition of claim 23, wherein said protein is glycosylated.

30. The crystalline protein composition of claim 23, wherein said protein is deglycosylated.

31. The crystalline protein composition of claim 23, wherein said composition further includes a β-secretase substrate or inhibitor molecule.

32. The crystalline protein composition of claim 31, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 78 (VMXVAEF; P3-P4'X D->V), including conservative substitutions thereof.

33. The crystalline protein composition of claim 31, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 [P10-P4'sta D->V], including conservative substitutions thereof.

34. The crystalline protein composition of claim 31, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 81 [EVMXVAEF], wherein X is hydroxyethylene or statine.

35. The crystalline protein composition of claim 31, wherein said β-secretase inhibitor is characterized by a K_i of no more than about 0.5 mM.

36. The crystalline protein composition of claim 31, wherein said β-secretase inhibitor is characterized by a K_i of no more than about 50 μM.

37. An isolated protein, comprising a polypeptide that (i) is fewer than about 450 amino acid residues in length, (ii) includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 75 [63-423] including conservative substitutions thereof, and (iii) exhibits β-secretase activity, as evidenced by an ability to cleave a substrate selected from the group consisting of the 695 amino acid isotype of beta amyloid precursor protein (ßAPP) between amino acids 596 and 597 thereof, MBP-C125wt and MBP-C125sw.

38. The protein of claim 37, wherein said polypeptide includes the amino acid sequence of SEQ ID NO: 75 [63-423].

39. The protein of claim 37, wherein said polypeptide has the sequence SEQ ID NO: 75 [63-423].

40. The protein of claim 37, wherein said amino acid sequence is at least 95% identical to SEQ ID NO: 58 [46-452].

41. The protein of claim 40, wherein said polypeptide has the sequence SEQ ID NO: 58 [46-452].

42. The protein of claim 37, wherein said protein consists of a polypeptide having the sequence SEQ ID NO: 58 [46-452].

43. The protein of claim 37, wherein said protein consists of a polypeptide having the sequence SEQ ID NO: 74 [22-452].

44. The protein of claim 37, wherein said protein is expressed by a heterologous cell.

45. A composition comprising the protein of claim 37 and a β-secretase substrate or inhibitor molecule.

46. The composition of claim 45, wherein said β-secretase substrate is selected from the group consisting of MBP-C125wt, MBP-C125sw, APP, APPsw, and β-secretase-cleavable fragments thereof.

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47. The composition of claim 46, wherein said β-secretase-cleavable fragment is selected from the group consisting of ~~SEVKMDAEF~~ (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, ~~SEVKFDAEF~~, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF;

SEQ ID NO:82 - SEQ ID NO:96

SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

48. The composition of claim 45, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 78 (VM[X]VAEF, where X is hydroxyethlene or statine), including conservative substitutions thereof.

49. The composition of claim 48, wherein said ß-secretase inhibitor has the sequence SEQ ID NO: 81 (VM[X]VAEF, where X is hydroxyethlene or statine).

50. The composition of claim 45, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 (P10-P4'sta D->V), including conservative substitutions thereof.

51. The composition of claim 37, wherein said β-secretase inhibitor has a K_i of no more than about 1 μM.

52. The composition of claim 37, wherein said β-secretase inhibitor is labeled with a detectable reporter molecule.

53. An isolated mouse ß-secretase protein enzyme having the sequence SEQ ID NO: 65.

54. An antibody which binds specifically to a purified ß-secretase protein comprising a polypeptide that includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 75 [63-423] including conservative substitutions thereof, wherein said antibody further lacks significant immunoreactivity with a protein a sequence selected from the group consisting of SEQ ID NO: 2 [1-501] and SEQ ID NO: 43 [46-501].

55. The antibody of claim 54, wherein said antibody is reactive with a protein selected from the group consisting of SEQ ID NO: 66 [22-501], SEQ ID NO: 67 [58-501], SEQ ID NO: 69

[63-501], SEQ ID NO: 59 [1-452], SEQ ID NO: 74 [22-452], SEQ ID NO: 58 [46-452], SEQ ID NO: 68 [58-452] and SEQ ID NO: 70 [63-452].

56. An isolated nucleic acid, comprising a sequence of nucleotides that encodes a β-secretase protein that is at least 95% identical to a protein selected from the group consisting of SEQ ID NO: 66 [22-501], SEQ ID NO: 43[46-501], SEQ ID NO: 57 [1-419], SEQ ID NO: 74 [22-452], SEQ ID NO: 58 46-452], SEQ ID NO: 59 [1-452], SEQ ID NO: 60 [1-420], SEQ ID NO: 67 [58-501], SEQ ID NO: 68 [58-452], SEQ ID NO: 69 [63-501], SEQ ID NO: 70 [63-452],SEQ ID NO: 75 [63-423], and SEQ ID NO: 71 [46-419], or a complementary sequence of any of such nucleotides, and specifically excluding a nucleic acid encoding a protein having the sequence SEQ ID NO: 2 [1-501].

57. The isolated nucleic acid of claim 56, wherein said sequence of nucleotides encodes a protease having an amino acid sequence SEQ ID NO: 58 [46-452].

58. The isolated nucleic acid of claim 56, wherein said sequence of nucleotides encodes a protease having the sequence SEQ ID NO: 43 [46-501].

59. The isolated nucleic acid of claim 56, wherein said sequence of nucleotides encodes a protease having the sequence SEQ ID NO: 66 [22-501].

60. The isolated nucleic acid of claim 56, wherein said sequence of nucleotides encodes a protease having the sequence SEQ ID NO: 74 [22-452].

61. A expression vector, comprising

the isolated nucleic acid of claim 56, and

operably linked to said nucleic acid, regulatory sequences effective for expression of the nucleic acid in a selected host cell.

62. The recombinant expression vector of claim 61, wherein said vector is suitable for transfection of a bacterial cell.

63. A heterologous cell transfected with the vector of claim 61, wherein said cell expresses a biologically active β-secretase.

64. The cell of claim 63, wherein said cell is a eukaryotic cell.

65. The cell of claim 63, wherein said cell is a bacterial cell.

66. The cell of claim 63, wherein said cell is an insect cell.

67. The cell of claim 63, wherein said cell is a yeast cell.

68. A method of producing a recombinant β-secretase enzyme, comprising culturing a cell according to claim 63 under conditions to promote growth of said cell, and subjecting an extract or cultured medium from said cell to an affinity matrix.

69. The method of claim 68, wherein said affinity matrix contains a β-secretase inhibitor molecule.

70. The method of claim 69, wherein said inhibitor molecule is SEQ ID NO: 72 [P10-P4'staD->V].

71. The method of claim 68, wherein said matrix contains an antibody characterized by an ability to bind β-secretase.

72. The method of claim 71, wherein said antibody is according to claim 55.

73. A heterologous cell, comprising

(i) a nucleic acid molecule encoding an active ß-secretase protein according to claim 55;

(ii) a nucleic acid molecule encoding a β-secretase substrate molecule; and

(iii) operatively linked to (i) and (ii), a regulatory sequence effective for expression of said nucleic acid molecules in said cell.

74. The cell of claim 73, wherein said nucleic acid encoding said β-secretase protein is heterologous to said cell.

75. The cell of claim 73, wherein both said nucleic acids encoding said β-secretase protein encoding said β-secretase substrate molecule are heterologous to said cell.

76. The cell of claim 73, wherein said β-secretase substrate molecule is selected from the group consisting of MBP-C125wt, MBP-C125sw, APPwt, APPsw, and ß-secretase cleavable fragments thereof.

77. The cell of claim 76, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

78. A method of screening for compounds that inhibit Aβ production, comprising contacting an isolated β-secretase polypeptide according to claim 37 with (i) a test compound and (ii) a β-secretase substrate, and selecting the test compound as capable of inhibiting Aβ production if said β-secretase polypeptide exhibits less β-secretase activity in the presence of said compound than in the absence of said compound.

79. The method of claim 78, wherein said active β-secretase polypeptide has a sequence selected from the group consisting of SEQ ID NO: 43 [46-501] and SEQ ID NO: 58 [46-452].

80. The method of claim 78, wherein said β-secretase polypeptide and said substrate are produced by a cell according to claim 73.

81. The method of claim 78, which further includes administering said test compound to a mammalian subject having Alzheimer's disease or Alzheimer's disease-like pathology, and selecting said compound as a therapeutic agent candidate if, following such administration, said subject maintains or improves cognitive ability or said subject shows reduced plaque burden.

82. The method of claim 81, wherein said subject is a mammalian species comprising a transgene.

83. The method of claim 81, wherein said subject is a mouse bearing a transgene which encodes a human ß-amyloid precursor protein (ß-APP), including a mutant variant thereof.

84. The method of claim 78, wherein said β-secretase substrate is selected from the group consisting of MBP-C125wt, MBP-C125sw, APP, APPsw, and ß-secretase-cleavable fragments thereof.

85. The method of claim 78, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4'wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

86. A method of screening for compounds that inhibit Aβ production, comprising measuring binding of a purified β-secretase polypeptide according to claim 1 with a β-secretase inhibitor compound in the presence of a test compound, and selecting the test compound as β-

secretase active-site binding compound, if binding of the inhibitor in the presence of said test compound is less than binding of the inhibitor in the absence of said test compound.

87. The method of claim 86, wherein said inhibitor compound is labeled with a detectable marker.

88. The method of claim 86, wherein said β-secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 78 (VM[X]VAEF, where X is hydroxyethlene or statine), including conservative substitutions thereof.

89. The method of claim 86, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 (P10-P4'sta D->V), including conservative substitutions thereof.

90. The method of claim 86, wherein said β-secretase inhibitor has a Ki with respect to ß-secretase of less than about 50 μM.

91. A β-secretase inhibitor compound selected according to the method of claim 78.

92. The inhibitor of claim 91, wherein said compound is selected from a phage display selection system.

93. The compound of claim 92, wherein the phage display selection system is biased for the sequence SEQ ID NO: 72 [P10-P4'D→V].

94. A β-secretase inhibitor compound selected according to the method of claim 81.

95. The inhibitor of claim 94, wherein said compound is selected from a phage display selection system.

96. The compound of claim 95, wherein the phage display selection system is biased for the sequence SEQ ID NO: 72 [P10-P4'D→V].

97. A β-secretase inhibitor compound selected according to the method of claim 86.

98. The inhibitor of claim 97, wherein said compound is selected from a phage display selection system.

99. The compound of claim 98, wherein the phage display selection system is biased for the sequence SEQ ID NO: 72 [P10-P4'D→V].

100. A β-secretase inhibitor, comprising a peptide containing the sequence SEQ ID NO: 78 (VM[X]VAEF, where X is hydroxyethlene or statine), including conservative substitutions thereof.

101. The β-secretase inhibitor of claim 100, having the sequence SEQ ID NO: 72 (P10-P4'sta D->V).

102. The β-secretase inhibitor of claim 100, having the sequence SEQ ID NO: 78.

103. The β-secretase inhibitor of claim 100, having the sequence SEQ ID NO: 81.

104. A screening kit, comprising

an isolated β-secretase protein according to claim 37,

a cleavable β-secretase substrate, and

means for detecting cleavage of said substrate by β-secretase.

105. The screening kit of claim 104, wherein said β-secretase protein is present in a heterologous cell.

106. The screening kit of claim 104, wherein said β-secretase substrate molecule is selected from the group consisting of MBP-C125wt, MBP-C125sw, APPwt, APPsw, and β-secretase cleavable fragments thereof.

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107. The screening kit of claim 106, wherein said β-secretase-cleavable fragment is selected from the group consisting of SEVKMDAEF (P5-P4' wt), SEVNLDAEF (sw), SEVKLDAEF, SEVKFDAEF, SEVNFDAEF, SEVKMAAEF, SEVNLAAEF, SEVKLAAEF; SEVKMLAEF, SEVNLLAEF, SEVKLLAEF, SEVKFAAEF, SEVNFAAEF, SEVKFLAEF, and SEVNFLAEF.

108. A knock-out mouse, characterized by inactivation or deletion of an endogenous ß-secretase gene.

109. The knock-out mouse of claim 108, wherein said ß-secretase gene encodes a protein having at least 90% sequence identity to the sequence SEQ ID NO: 65.

110. The knock-out mouse of claim 108, wherein said deletion is inducible.

111. The knock-out mouse of claim 110, wherein said inducible expression is effected by a Cre-lox expression system inserted into the mouse genome.

112. A method of screening for drugs effective in the treatment of Alzheimer's disease or other cerebrovascular amyloidosis characterized by Aβ deposition, comprising

administering to a mammalian subject characterized by overexpression of ß-APP and/or deposition of Aβ a test compound selected for its ability to inhibit β-secretase activity a ß-secretase protein according to claim 37, and

selecting the compound as a potential therapeutic drug compound, if it reduces the amount of Aß deposition in said subject or if it maintains or improves cognitive ability in said subject.

113. The method of claim 112, wherein said mammalian subject is a transgenic mouse bearing a transgene encoding a human ß-APP or a mutant thereof.

114. A method of treating a patient afflicted with or having a predilection for Alzheimer's disease or other cerebrovascular amyloidosis, comprising

blocking the enzymatic hydrolysis of APP to Aβ in the patient by administering to the patient a pharmaceutically effective dose of a compound effective to inhibit a β-secretase enzyme protein according claim 37.

115. The method of claim 114, wherein said compound is derived from a peptide selected from the group consisting of SEQ ID NO: 72, SEQ ID NO: 78, SEQ ID NO: 81 and SEQ ID NO: 97.

116. A method of inhibiting enzymatic proteolysis of APP to Aβ in a tissue, comprising contacting said tissue with a compound effective to inhibit the enzymatic activity of a β-secretase protein according to claim 37.

117. The method of claim 116, wherein said inhibition of enzymatic activity is evidenced by a K_i of less than about 50 μM in a MBP-C125sw assay.

118. A therapeutic drug composition for the treatment of Alzheimer's disease or other cerebrovascular amyloidosis characterized by deposition of Aβ peptide, wherein the active compound in said drug is selected for its ability to inhibit the enzymatic activity of a β-secretase protein according to claim 37.

119. The therapeutic drug of claim 118, wherein said inhibition of enzymatic activity is evidenced by a K_i of less than about 50 μM in a MBP-C125sw assay.

120. The therapeutic drug of claim 118, wherein said drug is derived from a peptide selected from the group consisting of SEQ ID NO: 72, SEQ ID NO: 78, SEQ ID NO: 81 and SEQ ID NO: 97.

121. A method of diagnosing the presence of or a predilection for Alzheimer's disease in a patient, comprising

detecting the expression level of a gene comprising a nucleic acid encoding ß-secretase in a cell sample from said patient, and

diagnosing the patient as having or having a predilection for Alzheimer's disease, if said expression level is significantly greater than a pre-determined control expression level.

122. The method of claim 121, wherein said gene comprises a nucleic acid that encodes a β-secretase protein that is at least 95% identical to a protein selected from the group consisting of SEQ ID NO: 66 [22-501], SEQ ID NO: 43[46-501], SEQ ID NO: 57 [1-419], SEQ ID NO: 74 [22-452], SEQ ID NO: 58 46-452], SEQ ID NO: 59 [1-452], SEQ ID NO: 60 [1-420], SEQ ID NO: 67 [58-501], SEQ ID NO: 68 [58-452], SEQ ID NO: 69 [63-501], SEQ ID NO: 70 [63-452],SEQ ID NO: 75 [63-423], and SEQ ID NO: 71 [46-419], or a complementary sequence of any of such nucleotides.

123. The method of claim 121, wherein said nucleic acid specifically excludes a nucleic acid encoding a protein having the sequence SEQ ID NO: 2 [1-501].

124. The method of claim 121, wherein said nucleic acid encodes a protein having the sequence SEQ ID NO: 2 [1-501].

125. The method of claim 121, wherein said measuring is carried out in a whole cell assay.

126. The method of claim 109, wherein said measuring is carried out on a nucleic acid derived from a cell sample of said patient.

127. A method of purifying a β-secretase protein enzyme molecule, comprising

contacting an impure sample containing β-secretase enzyme activity with an affinity matrix which includes a β-secretase inhibitor.

128. The method of claim 127, wherein said β-secretase inhibitor comprises a peptide having the sequence SEQ ID NO: 78 (VM[X]VAEF, where X is hydroxyethlene or statine), including conservative substitutions thereof.

129. The method of claim 128, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 72 (P10-P4'sta D->V).

130. The method of claim 128, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 78.

131. The method of claim 128, wherein said β-secretase inhibitor has the sequence SEQ ID NO: 81.

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